IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE:	U.S.S.N. 09/397,110	)	
		)	
FILED:	September 16, 1999	)	
		)	
TITLE:	PROCESS AND MATERIALS FOR THE RAPID DETECTION OF *STREPTOCOCCUS PNEUMONIAE* EMPLOYING PURIFIED ANTIGEN-SPECIFIC ANTIBODIES	)	GROUP ART UNIT: 1645
		)	
		)	EXAMINER:
INVENTORS:	Norman James Moore, Mary K. Fent, Vladimir A. Koulchin Elena Valentin Molokova	)	Virginia Allen Portner

RESPONSE TO OFFICE ACTION
MAILED APRIL 15, 2003

Applicants hereby respond to the Office Action mailed April 15, 2003 as follows:

RESPONSE TO CLAIM REJECTIONS UNDER 35 U.S.C. §112

Claim 50 is herewith amended to recite, as it should ,that the "window" is in the housing and that the second zone portion of the bibulous ICT strip, in which a colored line appears when the target antigen is present in the sample, is positioned under the window within the housing. In making this amendment, a substantial amount of extraneous language has been omitted in an effort to make the claim clearer overall and easier to read.

It is submitted that, as now amended, the claim is no longer subject to a §112 rejection.

RESPONSE TO SECTION 103(a)
REJECTIONS

The rejection of Claims 50-51 under 35 U.S.C. §103(a) as obvious over May in view of Sundberg Kovanees *et al* (1996) in light of Freisen *et al* is traversed.

The pneumococcal C-polysaccharide antigen studied by Sundberg Kovamees, Holme and Sjogren in the cited article is not the cell wall polysaccharide antigen common to *all* serotypes of *S. pneumoniae* which is detected by Applicant's ICT test. Instead, it is the usually capsular *species-specific* polysaccharide antigen of *S. pneumoniae*. This latter, species-specific, polysaccharide has been used in a vaccine which is referred to at p.224 of Sundberg-Kovamees, first full sentence on the page, as being of suboptimal efficacy. Proof that the Sundberg-Kovamees *et al* article is referring to the species-specific antigen clearly appears at p.232 where, under the heading "Test for binding of bacterial and soluble substances to immobilized receptors", it appears that the monoclonal anti-phosphoryl choline antibody and the polyclonal rabbit antipneumococcal antiserum used in the study described in the article are the *same antibodies,* respectively, as those utilized by the two Sundberg-Kovamees coauthors, Holmes and Sjogren, in the work reported upon in the article "A highly specific two-site ELISA for pneumococcal C-polysaccharide using monoclonal and affinity-purified polyclonal antibodies, J. Immunol. Methods, (1987) *102;* 93-100.

Reference to the latter Sjogren and Holme article shows that the polyclonal antibodies were obtained from a rabbit immunized with whole bacteria of *Streptococcus pneumoniae* type 1 strain "because purified capsular antigen is a poor antigen in rabbits (p.95, righthand column). The article states further that the antigen detected by the monoclonal antibody/polyclonal antibody two site ELISA, (referred to in *both articles* as "PnC" is a

species-specific cell wall component of pneumococci." See Sjogren and Holme p.94, first full sentence of righthand column.

The Sjogren article shows that the polyclonal antibodies obtained in rabbits that had been immunized with whole bacteria were obtained by running crude rabbit serum over PnC-coupled Sepharose (p.96, lefthand column) and that this purification "resulted in a preparation of antibodies reacting only with capsular antigen and with PnC" (p.95, left hand column, first *full* paragraph). Taken with the definition of PnC as "species-specific" (p.94, right hand column) all of this suggests that the two site assay in both Sjogren and Sundberg-Kovamees *is* species-specific and would accordingly be of limited use as an assay for detecting *S. pneumoniae*-caused pneumococcal disease because a different antibody combination would be needed for each serogroup or strain of *S. pneumoniae*.

The cell-wall polysaccharide antigen detected in Applicant's ICT assay is *not* species-specific; it is found in *all* strains or serotypes of *S. pneumoniae*. The ability of the assay to detect this antigen common in all strains or serotypes of *S. pneumoniae* is one of the major virtues which has played an important part in its FDA approval in 2000 and its world wide clinical acceptance since that time.

While May *et al* show a specific device which could be modified to run Applicants' assay, and Sundberg-Kovanees *et al may* show a test that could perhaps be adapted to run on the May *et al* device, the combination would not produce the device adapted to run Applicants' assay that is covered by Claims 50 and 51. Specifically, the combination of May *et al* and Sundberg-Kovamees would most likely produce a test device wherein the labelled antibodies deposited in a first zone would be polyclonal and affinity purified and the immobilized antibodies striped on the second zone would be monoclonal, wholly *unlike* Claim

Applicants' assay differs markedly from the one shown by Sjogren *et al* and Sundberg-Kovamees in that identical antibodies react with the target antigen at *both* of its determinants.

35 U.S.C §103(a) requires that the reference combination cited be of a character whereby the differences between the combined teachings and the invention are such that "the invention *as a whole* would have been obvious to a person of ordinary skill" from the combined teachings. The Sundberg-Kovamees assay, as further illuminated by the Sjogren *et al* article as to the polyclonal antibody preparation employed, simply contains too many differences in the antibodies from applicant's purified antigen-specific antibodies and, overall, seems clearly to be directed at a *different* target *S. pneumoniae* antigen from the one detected in Applicants' ICT assay so that its end use would be specific and limited rather than inclusive and broadly effective as Applicants' assay is.

Withdrawal of the rejection is accordingly deemed appropriate and is hereby requested.

CLAIM LISTING

1 (Withdrawn) A method for obtaining a cell wall C-polysaccharide antigen containing not more than about 10% protein from the bacterium *Streptococcus pneumoniae* which comprises the steps of:

(a) culturing th bacterium for a time requisite to obtain a sample of desired size and harvesting the bacterial cells therefrom in the form of a wet cell pellet;

(b) suspending the wet cell pellet in an alkaline solution and mixing;

(c) adjusting the pH to an acid pH with a strong acid and centrifuging;

(d) separating the supernatant from step (d) and adjusting its pH to approximate neutrality;

(e) digesting this product with a broad spectrum protease enzyme preparation to destroy residual proteins;

(f) adjusting the pH to the alkaline side with a weakly alkaline aqueous solution

(g) separating out the essentially protein free [carbohydrate or] polysaccharide antigen on a size exclusion column equilibrated with a weakly alkaline solution; and

(h) pooling material eluted in the first peak and adjusting its pH to approximate neutrality.

2 (Withdrawn) The cell wall C-polysaccharide antigen containing not more than about 10% protein obtained by the method of claim 1.

3 (Withdrawn) A method according to claim 1 in which the alkaline solution of step (b) comprises about 20 ml. Per gram of said wet cell pellet of 0.1M aqueous sodium hydroxide.

4 (Withdrawn) A method according to claim 1 in which in step (c) the pH is adjusted to about 3.0.

5 (Withdrawn) A method according to claim 1 in which, instep (f) the pH is adjusted to a pH between about 10 and about 11.

6 (Withdrawn) A method according to claim 1 in which, afer step (h), a lyophilization step is performed.

7 (Withdrawn) A method for the purification of raw antibodies to *S. pneumoniae* which comprises the step of:

(a) separating from *S. pneumoniae* bacteria a cell-wall C-polysaccharide antigen containing not more than about 10%

(b) conjugating said antigen to one end of a two-ended spacer molecule to form a conjugate of said antigen with the spacer molecule;

8 (Withdrawn) Purified antigen-specific antibodies to the cell wall C-polysaccharide of *S. pneumoniae* obtained by the method of claim7.

9 (Withdrawn) A chromatographic column for affinity purification of raw antibodies to *S. pneumoniae* having coupled thereto by a spacer molecule a purified C-polysaccharide cell wall antigen of *S. pneumoniae* containing not more than about 10% protein.

10-32 Cancelled

33 (Previously Presented) A method of detecting the presence of the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, in a liquid sample, which method comprises the following steps:

a) culturing *Streptococcus pneumoniae* bacteria, to obtain a desired size of culture and harvesting therefrom cells thereof as a wet cell pellet;

b) separating from the wet cell pellet the cell wall C-polysaccharide antigen containing not more than 10% protein by performing a series of steps which comprises;

(i) suspending the wet cell pellet in an alkaline solution and mixing;

(ii) adjusting the pH to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the product from step (v) to an alkaline pH with a weakly alkaline aqueous solution; and (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;

c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen containing not more than 10% protein obtained in step (b);

d) passing polyvalent antibodies to *Streptococcus pneumoniae* over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

e) conducting an immuno-assay upon a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its C-polysaccharide cell wall antigen which immuno-assay comprises the steps of

(i) contacting the liquid sample with conjugates of purified antigen specific antibodies from step(d) hereof and a labelling agent capable of manifesting a color or a detectable signal upon completion of the immunoassay, whereupon C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in the sample, whether or not in free form, will react with said conjugates to form labelled antibody-antigen conjugates,

(ii) further contacting the liquid and all of the conjugates it contains with a solid surface upon which a mass of unlabelled antigen-specific antibodies from step (d) hereof have been immobilized, whereupon any labelled antibody-antigen conjugates present will react with the immobilized antibodies on the surface to form labelled antibody-antigen-immobilized antibody sandwiches, and

(iii) detecting any label thereby accumulated on the solid surface by a detection means appropriate to the nature of the label so as to confirm the presence of the *Streptococcus pneumoniae* C-polysaccharide cell wall antigen in the sample.

34 (Previously Presented) The method of claim 33 in which the spacer molecule of step (c) is a protein molecule.

35 (Previously Presented) The method of claim 33 wherein the sample of step (e) is a natural liquid of mammalian origin.

36 (Previously Presented) The method of claim 35 wherein the liquid sample of step (e) is human urine.

37 (Previously Presented) The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of pneumonia.

38 (Previously Presented) The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

39 (Previously Presented) The method of claim 35 wherein the liquid sample of step (e) is human spinal fluid.

40 (Previously Presented) The method of claim 39 wherein the sample is obtained from a patient suspected of having meningitis.

41 Cancelled

42 (Previously Presented) The method of claim 33 in which step (e) is an immunochromatographic ("ICT") process.

43 (Previously Presented) The method of claim 42 in which step (e) is conducted by

a) contacting a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its free cell wall C-polysaccharide antigen, with the sample-receiving end of a strip of bibulous material, which strip is contained within an ICT device comprising a housing and itself comprises

(i) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) of claim 33, said labelling agent being selected from among those known to manifest a visible color change upon the formation of a labelled antibody - antigen - fixed antibody reaction product and

(ii) a second zone having fixedly bound thereto a stripe of unconjugated purified antigen-specific antibodies from step (d) of claim 33, which zone is equipped with a window in the housing for viewing the appearance of a color characteristic of the massing of the labelling agent upon the formation of the labelled antibody - antigen - fixed antibody reaction product;

b) allowing said liquid sample to flow laterally along said test strip to said first zone where it picks up the movably embedded conjugate of labelling agent and antigen-specific antibodies obtained in step(d) of Claim 33

c) allowing said liquid sample and said conjugate of antigen-specific antibodies to flow laterally together along said test strip to said second zone while concomitantly reacting to form labelled antibody-antigen conjugates with C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, free or combined, present in the sample and

d) within not more than 20 minutes after first contacting the liquid sample with the test strip, observing, through said window in the housing whether a line of color has formed, indicative of the massing of said label along the stripe of unconjugated purified antibodies, as labelled antibody-antigen-fixed antibody reaction products are formed.

44 (Previously Presented) The method of claim 43 wherein the sample is a natural liquid of mammalian origin.

45 (Previously Presented) The method of claim 44 wherein the sample is human urine.

46 (Previously Presented) The method of claim 45 wherein the sample is taken from a patient exhibiting overt clinical signs of pneumonia or another respiratory tract illness known to be often caused by *Streptococcus pneumoniae*.

47 (Previously Presented) The method of claim 44 wherein the liquid sample is human spinal fluid.

48 (Previously Presented) The method of claim 45 wherein the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

49 (Previously Presented) The method of claim 45 wherein the liquid sample is taken from a patient suspected of having meningitis.

50 (Currently amended) An ICT device for the detection of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in a liquid sample, which device comprises a housing [equipped with a window and] containing a strip of bibulous material ~~so positioned that its second zone~~ having [at least a first zone

and a second zone, said strip being so positioned within said housing that its second zone appears directly beneath said window, said strip being further characterized in that]

a) [said first zone has] ~~a first zone in which has been~~ movably embedded [therein] a conjugate of a labeling agent and purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, and

b) [said second zone is located] ~~a second zone~~ downstream of said first zone ~~having~~ [and has] immovably bound thereto a stripe of purified antibodies specific to the same cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, ~~which zone is equipped with a window in the housing for viewing the appearance of a line of color along said stripe, which color is indicative of the massing of the labelling agent along the immovably bound stripe as a consequence of the formation of labelled antibody-antigen-immovable antibody sandwiches, whereby the line of color denotes the presence in the liquid sample of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*;~~

all of which antibodies in both zones are further characterized in that their antigen specificity has been attained by passing polyvalent antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled ~~a spacer~~

~~molecule conjugated to~~ a purified cell wall C-polysaccharide antigen obtained from a culture of *Streptococcus pneumoniae* bacteria according to the following method:

(i) harvesting cells from the said culture in the form of a wet cell pellet;

(ii) suspending the wet cell pellet in an alkaline solution and mixing;

(iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;

(iv) separating the acidified product from step (iii) into two layers;

(v) removing the upper layer and adjusting its pH to approximate neutrality;

(vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and

(viii) separating out the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* having no more than 10% protein.

51 (Previously Presented) The ICT device of claim 50 wherein the labelling agent is finely divided metallic gold.

52-54 Cancelled